Mercury Concentrations in Gonad, Liver, and Muscle of White Sturgeon, *Acipenser transmontanus*, in the Lower Columbia River

M. A. H. Webb[1*], G. W. Feist[1], M. S. Fitzpatrick[2], E. P. Foster[2], C. B. Schreck[3], M. Plumlee[4], C. Wong[4], and D. T. Gundersen[4]

[1] Department of Fisheries and Wildlife, 104 Nash Hall, Oregon State University, Corvallis, OR 97331 USA

[2] Oregon Department of Environmental Quality, Water Quality Division, 811 SW 6th Avenue, Portland, OR 97204 USA

[3] Oregon Cooperative Fish and Wildlife Research Unit, Biological Resources Division, US Geological Survey, Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331 USA

[4] Department of Environmental Science, 2043 College Way, Pacific University, Forest Grove, OR 97116, USA

[*] Corresponding Author: Tel: (541)737-2463, FAX: (541)737-3590, Email: Molly.Webb@oregonstate.edu

Abstract

This study determined the partitioning of total mercury in liver, gonad, and cheek muscle of white sturgeon (*Acipenser transmonatus*) in the lower Columbia River. The relationship between tissue mercury concentrations and various physiological parameters were assessed. White sturgeon were captured in commercial fisheries in the estuary and Bonneville, The Dalles, and John Day Reservoirs. Condition factor, relative weight, and gonadosomatic index were determined for each fish (n=57). Gonadal tissue was examined histologically to determine sex and stage of maturity. Liver (n=49), gonad (n=49), and cheek muscle (n=57) were analyzed for total mercury using cold vapor atomic fluorescence spectrophotometry. Tissue protein concentrations were measured by UV-VIS spectroscopy. Plasma was analyzed for testosterone, 11-ketotestosterone, and estradiol-17ß using radioimmunoassay. Mean tissue mercury concentrations were higher in muscle compared to liver and gonad at all sampling locations, except Bonneville Reservoir where mean liver mercury content was the highest tissue concentration observed in the study. Significant negative correlations between plasma androgens (testosterone and 11-ketotestosterone) and muscle mercury content and plasma estradiol-17ß and liver mercury content were found. A significant positive linear relationship between white sturgeon age and liver mercury concentrations was evident. Significant negative correlations between condition factor and relative weight and gonad and liver mercury content were found. In addition, immature males with elevated gonad mercury content had reduced gonadosomatic indices. These results suggest that mercury, in the form of methylmercury, may have an effect on the reproductive potential of white sturgeon.

Introduction

Mercury is a non-essential metal in vertebrates that is embryotoxic, teratogenic and can affect behavior, biochemistry, growth, reproduction, development, and survival (Sorensen 1991, Wiener and Spry 1996). Mercury exists in many forms in the aquatic environment including elemental mercury, dissolved and particulate ionic forms, and dissolved and particulate methylmercury (Morel et al. 1998). Anthropogenic activities and natural geochemical processes determine the chemical forms and concentration of mercury in the aquatic environment (Weiner and Spry 1996). In fishes, 95 to 99% of the mercury is present in the form of methylmercury (Bloom 1992, Handy 1996), and approximately 90% of the methylmercury that accumulates in wild fish occurs through dietary uptake (Spry and Wiener 1991; Wiener and Spry 1996).

Little information is available regarding the toxicological effects of methylmercury on reproduction of wild fishes. Many laboratory experiments have exposed fish to water-borne mercury or dietary mercury at concentrations that are not environmentally relevant (μg/L). Mercury levels in natural waters in the Northern Hemisphere typically do not exceed the ng/L range (Weiner and Spry 1995). In laboratory experiments using environmentally relevant concentrations, mercury has been found to delay spawning, reduce gonadosomatic index (GSI), and suppress levels of circulating sex steroids (e.g., Matta et al. 2001, Hammerschmidt et al. 2002, Drevnick and Sandheinrich 2003).

Tissue concentrations of methylmercury in exposed fish are often greatest in the blood, spleen, kidney, and liver (see Wiener and Spry 1996). However, internal redistribution of methylmercury to muscle can occur due to the high affinity of methlymercury for protein sulfhydryl groups (Boudou and Ribeyre 1983, Harrison et al. 1990). Factors that affect accumulation of methylmercury in fishes include biomagnification (Handy 1996, Weiner and

Spry 1996), age (Weiner and Spry 1996), anthropogenic inputs (Kime 1995), and atmospheric deposition (Kime 1995).

The Columbia River in the Pacific Northwest, USA, is subject to pollution from a variety of sources including paper and pulp mills, smelters, and mining as well as natural weathering processes that may contribute mercury to the aquatic environment. The lower Columbia River (defined as the estuary below Bonneville Dam, Bonneville Reservoir, The Dalles Reservoir, and John Day Reservoir in this study) supports both commercial and sport fisheries for consumption of white sturgeon (*Acipenser transmontanus*), and impounded populations have lower recruitment compared to the unimpounded population (Beamesderfer et al. 1995, DeVore et al. 1995). White sturgeon are a long lived, late maturing species that are benthically-oriented. Because of these life history characteristics, sturgeon are highly susceptible to bioaccumulation of persistent environmental contaminants such as mercury.

The objectives of this study were to quantitatively determine the partitioning of total mercury in liver, gonad, and cheek muscle of white sturgeon in the lower Columbia River and assess the relationship between tissue mercury concentrations and various physiological parameters, including plasma sex steroids, age, condition factor (CF), relative weight (W_r) and GSI.

Materials and Methods

Tissue Collection

Tissue samples (liver, gonad, cheek muscle) and blood were collected from white sturgeon during the commercial harvest in February-April of 2000 and 2001 from the estuary and three lower Columbia River reservoirs (Bonneville, The Dalles, and John Day Reservoirs).

Cheek muscle in sturgeon is red muscle and is herein referred to as muscle. Table 1 shows the number of fish samples from each site analyzed for mercury. The legal size slot limit for commercial harvest in the lower Columbia river is 110-137 cm fork length. Length (± 0.5 cm) and weight (± 1 kg) were recorded, and CF and W_r were determined for each fish. Gonads were removed, weighed (± 0. 0001 g), and GSI was determined. In 2000, tissue samples were wrapped in aluminum foil and stored at -40°C until analyzed for mercury content. In 2001, tissue samples were placed in I-Chem environmental sample glass jars (Type III, Fisher) and stored at -40°C until analysis. Blood was collected from the caudal vasculature using a heparinized vacutainer, centrifuged, and plasma was stored at -80°C until sex steroid concentrations were determined by radioimmunoassay (RIA). A subsample of gonadal tissue was collected for histological analysis. In 2001, pectoral fin spines were collected from a subsample (n=44) of sturgeon for age determination.

Tissue samples (liver, fully grown ovarian follicles, muscle, fillet) and blood were collected from a single mature female in Bonneville Reservoir in May 2003. The tissue samples were placed in I-Chem glass jars and stored at -40°C until analysis. Blood was collected and plasma stored as described above. Length, weight, and GSI were determined, and a fin spine was collected for age determination. Six subsamples of ovarian follicles were collected (n=3 from each lobe of the gonad) and weighed (± 0.0001 g) to estimate fecundity.

Mercury analysis

Digestion procedures of sturgeon tissues for total mercury were done based on the methods described by Gloss et al. (1990). Analysis of digested tissues was done according to U.S. EPA method 1631, revision B (1999). Subsamples (~ 1 g) of homogenized sturgeon tissues

were digested in concentrated sulfuric acid (70ºC) for 30 min. Then, 30% hydrogen peroxide was added and digestion was continued for 2 additional hours. Potassium permanganate (5%) was added to digested samples to oxidize the tissue mercury followed by dilution with distilled water (100 mL total volume). Samples were stored in capped containers at 4ºC until later analysis.

An aliquot (0.5 to 1.0 mL) of the digested samples was added to glass bubblers containing 50 mL of distilled water, 5 mL of a hydroxylamine hydrochloride solution (300g/L) and 2 mL of a 10% stannous chloride solution. Bubblers were purged with ultra-pure nitrogen for 20 min, and the elemental mercury was collected on gold-coated quartz grains. The trapped mercury was subsequently desorbed thermally and measured on a Tekran Cold Vapor Atomic Fluorescence Spectrophotometer (CVAFS, Model 2500). Peak areas were recorded using a Hewlett Packard 3396A Integrator. Working mercury standards were prepared from a stock mercury standard containing 1000 µg Hg/L in nitric acid (Sigma-Aldrich St. Louis, MO). Quality assurance measures included the analysis of blanks, duplicates, and spiked samples (for each batch of 10 samples). Percent recovery of spiked samples ranged from 80 to 101%. The method detection limit was 17 ppb. All sample concentrations are reported on a wet weight basis. Protein concentrations were measured using the Lowry-Bronsted method (Lowry et al., 1951), and the sample absorbance was measured by UV-VIS spectroscopy.

Radioimmunoassays

The steroids, testosterone (T), 11-ketotestosterone (KT), and 17ß-estradiol (E2), were extracted from plasma following the method of Fitzpatrick et al. (1986). Extraction efficiencies for all steroids were determined by adding tritiated steroids to tubes containing plasma (n=4),

which were extracted as described above. The average extraction efficiencies for T, KT, and E2 were 91, 83, and 81%, respectively. All steroid assay results were corrected for recovery.

Plasma concentrations of T, KT, and E2 were measured by RIA as described in Sower and Schreck (1982) and modified by Feist et al. (1990). The lower limit of detection was 1.25 pg/tube for all assays, except KT (3.12 pg/tube). The intra- and inter-assay coefficients of variation for all assays were less than 5 and 10%, respectively. Steroid levels determined by RIA were validated by verifying that serial dilutions were parallel to standard curves.

Histology

Gonadal tissue was stored in 10% phosphate-buffered formalin, embedded in paraffin, sectioned at 7 µm, and stained by hematoxylin and eosin (Luna, 1968). Slides were examined under a compound scope (Motic, 10x-100x). Germ cells were scored for stage of development according to the protocol of Van Eenennaam and Doroshov (1998). Stage 1 (differentiation of testis and ovary) and Stage 2 (proliferation of spermatogonia and endogenous growth of the oocyte) fish were immature, while Stage 3 - 6 males (onset of meiosis through spermiation) were classified as maturing.

Age Determination

Ages of white sturgeon were estimated from thin cross-sections of pectoral fin spines following the procedures described in Beamesderfer et al. (1989) by Oregon Department of Fish and Wildlife (ODFW) personnel. Each fin spine section was aged by Ruth Farr and Michele Hughes at ODFW and by Joel Van Eenennaam and Javier Linares-Casenave at the University of California, Davis. The percentage of fish with identical age assignments was 20%. The

percentage of fish aged within 1 year by the different readers was 71%, within 2 years was 43%, within 3 years was 3%, and > 5 years was 6%. Similarly, Rien and Beamesderfer (1994) reported white sturgeon age assignments among experienced readers were 37% identical (68% within 1 year, and 83% within 2 years): both accuracy and precision of age estimates declined in older fish. We averaged the ages of the four readers for analyses.

Condition Factor and Relative Weight

Condition factor and W_r from paired measures of length and weight were estimated. The relative weight index is commonly used by fisheries biologists because it allows comparison among populations and among individuals of widely different sizes. A fish with a W_r of 100% matches the 75^{th}-percentile weight for fish of the same 1-cm length throughout the species range (Beamesderfer 1993).

Statistical Analyses

Comparisons between tissue protein concentrations, sex, plasma steroid concentrations, age, CF, W_r, GSI, river location, and tissue mercury content were conducted using a one-way ANOVA with the Bonferroni procedure. Correlations between tissue mercury load and plasma sex steroid concentrations were conducted using reciprocal-Y regression (reciprocal transformation of Y variable). All other correlations between tissue mercury levels and physiological parameters were conducted using simple linear regression. The accepted level of significance for all tests was $\alpha = 0.05$, except where the value of α was adjusted using the Bonferroni procedure. All statistical tests were conducted using StatView 4.51 (Abacus Concepts, Inc.). Data reported are means ± sem.

Results

Legal-size sturgeon

Mercury tissue concentrations. In the lower Columbia River, mercury concentrations in white sturgeon, regardless of sex or sampling location, were significantly higher ($P < 0.0001$) in muscle (170.54 ± 12.67 ppb, n=57) and liver (140.26 ± 23.02 ppb, n=49) compared to gonadal tissue (27.26 ± 2.50 ppb, n=49). Mercury did not appear to partition in tissue in a sex-specific manner, i.e., concentrations were not consistently higher in females compared to males or vice versa. Because no sex-specific differences in tissue mercury content were found at each site, the mean tissue mercury levels were calculated using both male and female values (Figure 1). Liver mercury concentrations differed significantly between locations ($P < 0.0001$), and liver mercury content was found to be significantly higher in Bonneville Reservoir compared to the estuary, The Dalles Reservoir, and John Day Reservoir (Figure 1). Muscle mercury levels did not differ between locations ($P = 0.3650$) (Figure 1). Concentrations of mercury in gonadal tissue did differ significantly at the four locations ($P = 0.0006$) (Figure 1), with mercury concentrations in gonadal tissue significantly higher in Bonneville Reservoir compared to the estuary, The Dalles Reservoir, and John Day Reservoir.

Protein concentrations. Protein concentrations were greatest in liver (519.24 ± 28.30 µg/g) followed by muscle (469.57 ± 29.62 µg/g) and gonad (293.80 ± 19.77 µg/g) and differed significantly by tissue type ($P < 0.0001$). Liver and muscle protein concentrations were significantly greater compared to gonad levels. No differences in tissue protein concentrations were evident between sites or sexes.

Sex. All of the females (n = 26) were sexually immature (Stage 2). Two of the males were sexually mature (Stage 5) with spermatozoa present in the testicular cysts, while the remaining males (n = 29) were immature (Stage 2).

Plasma Sex Steroids. Sex-specific steroid concentrations were not detected nor did plasma steroid concentrations differ among sites within the females or males. Steroid concentrations were low in immature fish (Table 2), while androgen concentrations were elevated in the two ripe males (estuary: T = 150.43 ng/mL, KT = 123.69 ng/mL; The Dalles Reservoir: T = 88.62 ng/mL, KT = 56.03 ng/mL). Because sex- and site-specific steroid concentrations were not detected, regression analysis was conducted using all fish. Significant negative correlations between plasma T and KT concentrations and muscle mercury content (Figure 2) and plasma estradiol concentration and liver mercury content (Figure 2) were found. Removing the two mature males from the regression analysis of androgens and muscle mercury content did not change the R^2 or P values.

Spermatogonia proliferation (Stage 2) in white sturgeon is associated with increased circulating androgen concentrations regardless of age or size (Feist et al. 2004). In immature wild white sturgeon in the Columbia River, T concentrations $\geq$ 4 ng/mL were used to differentiate Stage 2 males from Stage 1 males and immature females (Webb et al. 2002). As all immature males in this study were in Stage 2 of gonadal development, this steroid value was chosen to examine the relationship between tissue mercury content and T production. Of the 29 immature males, 21 of these males had circulating concentrations < 4 ng/mL of T. No males with muscle, liver, and gonad total mercury levels above 186.86 ppb, 93.24 ppb, and 74.09 ppb respectively, had plasma T concentrations > 4 ng/ml (Figure 3).

Age. The age of fish captured within the legal-size slot limit in the commercial fisheries in the lower Columbia River ranged from 10 to 27 years old (17 ± 1 years old). Fish in Bonneville Reservoir were significantly older than fish in the estuary and The Dalles Reservoir but were not significantly older than fish in John Day Reservoir (Table 3). No statistical difference in age between females and males was found at each location. A significant positive linear relationship between the age of white sturgeon and liver mercury concentrations was found ($P = 0.0118$, $R^2 = 0.16$, Figure 4).

Condition Factor and Relative Weight. Condition factor and W_r of sturgeon differed significantly between the sampling locations in the lower Columbia River ($P < 0.0001$; Table 3). No statistical difference in CF nor W_r between females and males was found at each location. Both CF and W_r were significantly lower in sturgeon sampled in Bonneville Reservoir compared to the estuary and John Day Reservoir. Condition factor and W_r of sturgeon in The Dalles Reservoir did not differ significantly from sturgeon in Bonneville Reservoir. Significant negative correlations between CF and W_r and gonad and liver mercury concentrations were found (Figure 5). However, this relationship, was not observed when muscle mercury content was examined.

Gonadosomatic Index. The GSI was lowest in fish captured in Bonneville Reservoir compared to John Day Reservoir, the estuary, and The Dalles Reservoir (Table 3). No statistical differences were found between the fish captured at the four locations ($P = 0.2055$). The linear regression analysis did not reveal any significant correlations between GSI and tissue mercury content; however, the P value for the negative correlation between GSI and gonad mercury content was 0.0544. When the two mature males were removed from the data set, a significant linear relationship between GSI and gonad mercury content in all immature sturgeon was found

(Figure 6). The linear relationship between GSI and gonad mercury content appears to be driven by immature males ($P = 0.0122$, $R^2 = 0.26$), not immature females ($P = 0.0645$, $R^2 = 0.12$, data not shown).

Mature, over legal-size female

The size of the mature female from Bonneville Reservoir was 170.45 kg and 262 cm fork length. The gonad weight of this female was 45.91 kg, hence the GSI was 26.93%. The fecundity was estimated to be 1,560,940, and relative fecundity was 9,158/kg body weight. Age of this female was estimated to be 41 years. The tissue mercury concentrations in this female were 1,678 ppb in the liver, 1,094 ppb in the muscle, 28 ppb in the gonad, and 435 ppb in the fillet.

Discussion

In legal-size white sturgeon in the lower Columbia River, mean muscle mercury concentrations were (170.54 ± 12.67 ppb). Because methlymercury binds to sulfhydryl groups in protein, most methylmercury eventually accumulates in muscle (Wiener and Spry 1996). Comparatively, mean muscle mercury content of northern pike (*Esox lucius*) in Lake Champlain was found to be 325 ppb (Friedmann et al. 1996a). In New Jersey, mean muscle mercury content in largemouth bass (*Micropterus salmoides*) ranged from 300-5,420 ppb in three water bodies (Friedmann et al. 2002). In a survey of 154 Oregon streams and rivers, whole-fish mercury content in piscivores greater than 120 mm in length was 284 ppb (Peterson et al 2002). In Roosevelt Lake, a reservoir in the upper Columbia River, muscle mercury content in walleye ranged from 110-440 ppb (Munn and Short 1997). Schmitt and Brumbaugh (1990) reported a

mean nationwide body burden of freshwater fish in the US in 1984 of 100 ppb. Concentrations of muscle mercury in the range of 6-20 ppm have been found to adversely affect fishes (Weiner and Spry 1996). Although mercury concentrations for sturgeon are lower than reported for some fish species, sturgeon are at risk because they are longer lived than most freshwater fish and may accumulate higher levels of mercury throughout their life span.

Mean mercury concentrations were slightly higher in muscle of white sturgeon compared to liver, and concentrations in the muscle and liver were significantly higher compared to the level detected in gonadal tissue. The highest mean mercury content was detected in the liver of Bonneville Reservoir sturgeon (339.90 ± 63.42 ppb, Figure 1). Tissue concentrations of methylmercury in exposed fish are often greatest in the blood, spleen, kidney, and liver prior to internal redistribution of mercury into muscle (see Wiener and Spry 1996). Yet, muscle mercury concentrations were not significantly elevated in Bonneville Reservoir sturgeon compared to the other locations. Hence, one possible explanation for the elevated concentrations of mercury seen in the liver of Bonneville Reservoir sturgeon is that the liver of these fish is no longer capable of detoxification at a rate seen in the other three locations. It is also possible that these fish were recently exposed to mercury, and tissue redistribution had not yet occurred.

No sex-specific differences in tissue mercury concentrations were seen in white sturgeon, which is similar to the findings of Friedmann et al. (1996a) who found no sex-specific differences in northern pike. For largemouth bass (Lange et al. 1994), mercury tissue concentrations differed between sexes with faster-growing females having lower mercury concentrations compared to males. In four species of centrachids, muscle mercury content differed between sexes with females having higher mercury concentrations compared to males (Nicoletto and Hendricks 1988). The sex-specific tissue mercury content in the centrachids was

attributed to onset of reproduction, not differences in body size. Similarly, Sorensen (1991) found that females from seven of eight species examined in New York State that had higher muscle mercury levels compared to males. The white sturgeon in our study were of similar size and age (except for fish in Bonneville Reservoir) and most fish were immature as they were captured in commercial fisheries with a slot limit. It is possible that in older, larger sturgeon a sex-specific difference in tissue mercury concentrations may be found.

The negative correlations between plasma steroids and tissue mercury (T and KT vs. muscle mercury; E2 vs. liver mercury) suggest a possible adverse impact of environmental mercury on the reproductive physiology of Columbia River sturgeon. In fathead minnows (*Pimephales promelas*) exposed to dietary methylmercury at environmentally relevant concentrations, methylmercury suppressed circulating levels of T in males and E2 in females (Drevnick and Sandheinrich 2003). In other wild fish populations, body burden of heavy metals was found to be negatively correlated with plasma T in Arctic grayling (*Thymallus articus*) (Allen-Gil et al. 1993); however, in largemouth bass, plasma KT was positively correlated with muscle mercury content (Friedmann et al. 2002). The decrease in sex steroids in sturgeon with elevated muscle and liver mercury content suggests an increase in steroid clearance or a decrease in steroidogenesis. Neither mechanism was determined in this study. In rats, mercury was the most potent metal for suppression of steroidogenesis in Leydig cells in males (Ng and Liu 1990, Friedmann et al. 1998), and mercuric chloride inhibited the activity of 3ß-hydroxy-Δ^5-steroid dehydrogenase, an important enzyme involved in testosterone biosynthesis (Chowdhury et al. 1985). The data from this study suggest that threshold levels for liver, muscle, and gonad total mercury content may exist, above which the fish are incapable of elevating plasma T concentrations. Muscle and liver mercury concentrations may be better indicators of potential

adverse effects because methylmercury preferentially binds with sulfhydryl groups in protein and does not partition based on lipophilicity. Further work must be conducted to determine the specific effects of mercury on steroidogenesis in sturgeon and the level of T responsible for initiation of gonadal development and maturation.

The physiological result of decreased circulating sex steroids in white sturgeon in the Columbia River may be altered gametogenesis, a delay in sexual maturation, and/or reduced reproductive success. Androgens and estrogens are necessary for spermatogenesis and oogenesis (Schulz and Miura 2002, Patiño and Sullivan 2002). Testosterone is the precursor to E2, the steroid necessary for the onset of vitellogenesis in females (Patiño and Sullivan 2002). In males, E2 plays a role in spermatogonial renewal divisions (Amer et al. 2001, Schulz and Miura 2002), and androgens are required for spermatogenesis (Schulz and Miura 2002). As well, T, KT, and E2 play an important role in stimulation of the hypothalamo-pituitary-gonadal axis (see Okuzawa 2002). The slot limit in the commercial fisheries in the Columbia River has been set to target white sturgeon prior to first sexual maturation, hence further studies with older and larger fish would need to be conducted to determine if mercury is negatively impacting the onset of maturation and reproductive potential.

Muscle mercury concentrations have been found to increase with age (e.g., Sorensen 1991, Stafford and Haines 1997, Morel et al. 1998, Mauk and Brown 2001). In this study, a significant positive linear relationship between the age of white sturgeon and liver mercury concentrations was found. A relationship between age and muscle mercury content may not have been evident in this study due to the relatively narrow age range of fish sampled. Continued mercury accumulation throughout the life of fish appears possible in sturgeon as seen by the elevated concentration of mercury in muscle and relatively high concentration in the fillet of the

Bonneville Reservoir adult female. The concentration of mercury in the muscle of this female (1,094 ppb) exceeded the Environmental Protection Agency (300 ppb), Oregon Department of Human Health (350 ppb), and Food and Drug Administration (1,000 ppb) action limits for mercury in fish, while the mercury concentration in the fillet of this female (435 ppb) exceeded Environmental Protection Agency and Oregon Department of Human Health action limits.

Condition factor and W_r are often used to describe the overall health of a fish (Beamesderfer 1993, Moyle and Cech 1996). Several species of teleosts sampled from mercury-contaminated sites have been found to have low CF (Hontela et al. 1995, Munn and Short 1997). Significantly lower CF and W_r were found in white sturgeon in Bonneville Reservoir compared to the estuary and John Day Reservoir, though no significant differences were found between fish in Bonneville Reservoir and The Dalles Reservoir. Similar results have been described in the lower Columbia River by Beamesderfer et al. (1995) and C. Kern and T. Rien, Oregon Department of Fish and Wildlife unpublished data). The lower condition of sturgeon in the reservoirs, specifically Bonneville Reservoir, has been attributed to intraspecific competition (Beamesderfer et al. 1995). The negative correlations between CF and W_r and gonad and liver mercury content, as well as the elevated liver mercury concentrations seen in Bonneville Reservoir fish, suggest that contaminants must be considered when evaluating poor condition of the impounded sturgeon populations.

Gonadosomatic index has been found to be inversely related to tissue mercury content in several species of teleosts (both males and females, Kirubagaran and Joy 1992; males only, Friedmann et al. 1996b; females only, Hammerschmidt et al. 2002; females only, Drevnick and Sandheinrich 2003). In white sturgeon, a significant correlation between GSI and gonad mercury content was found only when the two mature males were removed from the data set (this is

probably due to the very large weight of the testes in these mature males (798.85 ± 129.05 g) compared to immature males (64.74 ± 9.04 g)). When the analysis was conducted on females and males separately, GSI of immature males and gonad mercury content was the only significant relationship found ($P = 0.0122$, $R^2 = 0.26$) similar to Friedmann et al. (1996b). The inhibition of gonadal development in males, as seen by a reduction in GSI, may be related to the suppression of sex steroid production and should be further investigated.

The concentrations of tissue mercury in white sturgeon in the lower Columbia River were on average higher in muscle and liver compared to gonad. The tissue mercury content in white sturgeon was correlated with suppressed circulating concentrations of sex steroids, reduced CF and W_r, and lower GSI in immature males. These results suggest that methylmercury may have an affect on the reproductive potential of white sturgeon.

Acknowledgments

This research was funded by a Science Research Initiation Grant from the Murdock Charitable Trust and U.S. Geological Survey, award no. 99HQAG0152. We would like to thank C. Anthony, R. Chitwood with the Oregon State University; T. Rien, R. Farr, M. Hughes with the Oregon Department of Fish and Wildlife; J. Linares-Casenavae , J. Van Eenennaam with the University of California, Davis; R. Bratton and J. and L. Olheiser with Gilmore Smokehouse; and S. Fick with Fishhawk Fisheries. C. Kern and T. Rien with Oregon Department of Fish and Wildlife provided historic relative weight information for white sturgeon in the reservoirs of the lower Columbia River, and T. Rien provided valuable comments on an earlier draft of this manuscript. We would like to thank Dr. Serge Doroshov for reviewing this manuscript.

References

Allen-Gil SM, Curtis L, Lasorra B, Crecelius E, Landers D (1993) Plasma testosterone as a sensitive biomarker to heavy metal exposure in feral arctic fish. Soc Environ Toxicol Chem, 14th Annual Meeting, Houston, TX, 14-18 November.

Amer MA, Miura T, Miura C, Yamauchi K (2001) Involvement of sex steroid hormones in the early stages of spermatogenesis in Japanese huchen (*Hucho perryi*). Biol Reprod 65:1057-1066.

Beamesderfer RCP, Elliot JC, Foster CA (1989) Report A. *In* Status and Habitat Requirements of White Sturgeon Populations in the Columbia River Downstream from McNary Dam (A.A. Nigro, Ed.). Annual Progress Report to Bonneville Power Administration, Portland, Oregon, pages 5-52.

Beamesderfer RC (1993) A standard weight (Ws) equation for white sturgeon. California Fish and Game 79:63-69.

Beamesderfer RCP, Rien TA, Nigro AA (1995) Dynamics and potential production of white sturgeon populations in three Columbia River reservoirs. Trans Am Fish Soc 124:857-872.

Bloom NS (1992) On the chemical form of mercury in edible fish and marine invertebrate tissue. Can J Fish Aquat Sci 49:1010-1017.

Boudou A, Ribeyre F (1983) Contamination of aquatic biocenoses by mercury compounds: an experimental ecotoxicological approach. In: Nriagu JO (ed) Aquatic Toxicology. John Wiley & Sons, NY, pp. 117-136.

Chowdhury AR, Vachhrajani KD, Chatterjee BB (1985) Inhibition of 3ß-hydroxy-Δ^5-steroid dehydrogenase in rat testicular tissue by mercuric chloride. Toxicol Letters 27:45-49.

DeVore JD, James BW, Tracy CA, Hale DA (1995) Dynamics and potential production of white sturgeon in the unimpounded Lower Columbia River. Trans Am Fish Soc 124:845-856.

Drevnick PE, Sandheinrich MB (2003) Effects of dietary methylmercury on reproductive endocrinology of fathead minnow. Environ Sci Technol 37:4390-4396.

Feist G, Schreck CB, Fitzpatrick MS, Redding JM (1990) Whole body sex steroid concentrations and gonadal histology in coho salmon during sexual differentiation. Gen Comp Endocrinol 80: 299-313.

Fitzpatrick MS, Van Der Kraak G, Schreck, CB (1986) Profiles of plasma sex steroids and gonadotropin in coho salmon, *Oncorhynchus kisutch*, during final maturation. Gen Comp Endocrinol. 62:437-451.

Friedmann AS, Watzin MC, Leiter JC, Brinck-Johnsen T (1996a) Effects of environmental mercury on gonadal function in Lake Champlain northern pike (*Esox lucius*). Bull Environ Contam Toxicol 56:486-492.

Friedmann AS, Watzin MC, Brinck-Johnsen T, Leiter JC (1996b) Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (Stizostedion vitreum). Aquat Toxicol 35:265-278.

Friedmann AS, Chen H, Rabuck LD, Zirkin BR (1998) Accumulation of dietary methylmercury in the testes of the adult brown Norway rat: impaired testicular and epididymal function. Environ Toxicol Chem 17:867-871.

Friedmann AS, Costain EK, MacLatchy DL, Stansley W, Washuta EJ (2002) Effect of mercury on general and reproductive health of largemouth bass (*Micropterus salmoides*) from three lakes in New Jersey. Ecotoxicol Environ Safety 52:11-122.

Gloss SP, Grieb TM, Driscoll CT, Schofield CL, Baker JP, Landers D, Porcella DB (1990) Mercury levels in fish from the upper peninsula of Michigan (ELS subregion 2B) in relation to lake acidity. EPA-600/3-90-068 U.S. Environmental Protection Agency, Washington, DC.

Hammerschmidt CR, Sandheinrich MB, Wiener JG, Rada RG (2002) Effects of dietary methylmercury on reproduction of fathead minnows. Environ Sci Technol 36:877-883.

Handy R (1996) Dietary exposure to toxic metals in fish In: Taylor E (ed) Toxicology of Aquatic Pollution. Cambridge University Press, NY, pp 29-33.

Harrison SE, Klaverkamp JF, Hesslein RH (1990) Fates of metal radiotracers added to a whole lake: accumulation in fathead minnow (*Pimephales promelas*) and lake trout (*Salvelinus namaycush*). Water Air Soil Pollut 52:277-293.

Hontela A, Dumont P, Duclos D, Fortin R (1995) Endocrine and metabolic dysfunction in yellow perch, *Perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence River. Environ Toxicol Chem 14:725-731.

Kime DE (1995) The effects of pollution on reproduction in fish. Rev Fish Biol Fish 5:52-96.

Kirubagaran R, Joy KP (1992) Toxic effects of mercury on testicular activity in the freshwater teleost, *Clarias batrachus* (L.). J Fish Biol 41:305-315.

Lange TR, Royals HE, Conner LL (1994) Mercury accumulation in largemouth bass (*Micropterus salmoides*) in a Florida Lake. Arch Environ Contam Toxicol 27:466-471.

Lowry OH, Rosebrough NJ, Fan AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275.

Luna LG (1968) Manual of Histological Staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill Book Co., NY, 258 pp.

Matta MB, Linse J, Cairncross C, Francendese L, Kocan RM (2001) Reproductive and transgenerational effects of methylmercury or Aroclor 1268 on *Fundulus heteroclitus*. Environ Toxicol Chem 20:327-335.

Mauk RJ, Brown ML (2001) Selenium and mercury concentrations in brood-stock walleye collected from three sites on Lake Oahe. Arch Environ Contam Toxicol 40:257-263.

Morel FMM, Kraepiel AML, Amyot M (1998) The chemical cycle and bioaccumulation of mercury. Annu Rev Ecol Syst 29:543-566.

Moyle PB, Cech JJ Jr (1996) Fishes: An Introduction to Ichthyology. Prentice Hall, Upper Saddle River, NJ 590 pp.

Munn MD, Short TM (1997) Spatial heterogeneity of mercury bioaccumulation by walleye in Franklin D. Roosevelt Lake and the upper Columbia River, Washington. Trans Am Fish Soc 126:477-487.

Nicoletto PF, Hendricks AC (1988) Sexual differences in accumulation of mercury in four species of centrachid fishes. Can J Zool 66:944-949.

Ng TB, Liu WK (1990) Toxic effect of heavy metals on cells isolated from the rat adrenal and testis. In Vitro Cell Dev Biol 26:24-28.

Okuzawa K (2002) Puberty in teleosts. Fish Physiol Biochem 26:31-41.

Patiño RP, Sullivan CV (2002) Ovarian follicle growth, maturation, and ovulation in teleost fish. Fish Physiol Biochem 26:57-70.

Peterson SA, Herlihy AT, Hughes RM, Motter KL, Robbins JM (2002) Level and extent of mercury contamination in Oregon, USA, lotic fish. Environ Toxicol Chem 21:2157-2164.

Rien, TA, RC Beamesderfer (1994) Accuracy and precision in age estimates of white sturgeon from pectoral fin rays. Trans Am Fish Soc 123:255 265.

Schmitt CJ, Brumbaugh WG (1990) National contaminant biomonitoring program: concentrations of arsenic, cadmium, copper, lead, mercury, selenium, and zinc in U.S. freshwater fish, 1976-1984. Arch Environ Contam Toxicol 19:731-747.

Schulz RW, Miura T (2002) Spermatogenesis and its endocrine regulation. Fish Physiol Biochem 26:43-56.

Sorensen EM (1991) Metal Poisoning in Fish. CRC Press, Boca Raton, Florida, 374 pp.

Sower SA, Schreck CB (1982) Steroid and thyroid hormones during sexual maturation of coho salmon (*Oncorhynchus kisutch*) in saltwater or freshwater. Gen Comp Endocrinol 47:42-53.

Spry DJ, Wiener JG (1991) Metal bioavailability and toxicity to fish in low-alkalinity lakes: a critical review. Environ Pollut 71:243-304.

Stafford CP, Haines TA (1997) Mercury concentration in Maine sport fishes. Trans Am Fish Soc 126:144-152.

U.S. Environmental Protection Agency (1999) Method 1631, Revision B: Mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry. EPA-821-R-99-005 Washington DC.

Van Eenennaam JP, Doroshov SI (1998) Effects of age and body size on gonadal development of Atlantic sturgeon. J Fish Biol 53:624-637.

Wiener JG, Spry DJ (1996) Toxicological significance of mercury in freshwater fish In: Beyer WN, Heinz GH, Redmon-Norwood AW (eds) Environmental Contaminants in Wildlife Interpreting Tissue Concentrations. CRC Lewis Publishers, Boca Raton, FL, pp 297-339.

Table 1. Number of tissue samples analyzed for mercury content from legal-size white sturgeon captured in commercial fisheries in the lower Columbia River estuary and reservoirs.

Location	Female	Male
Liver		
Estuary	6	7
Bonneville	6	6
The Dalles	6	6
John Day	6	6
Muscle		
Estuary	7	9
Bonneville	6	9
The Dalles	7	6
John Day	6	7
Gonad		
Estuary	6	7
Bonneville	6	6
The Dalles	6	6

John Day	6	6

Table 2. Sex steroid concentrations (ng/mL) in immature legal-size white sturgeon captured in commercial fisheries in the lower Columbia River estuary and reservoirs. Two mature males were excluded from the calculation of mean ± sem (n).

Location	Testosterone	11-Ketotestoserone	Estradiol
Females			
Estuary	1.27 ± 0.34 (7)	1.43 ± 0.22 (7)	0.07 ± 0.03 (7)
Bonneville	4.68 ± 2.87 (6)	2.23 ± 0.94 (6)	0.04 ± 0.02 (6)
The Dalles	0.66 ± 0.19 (7)	0.63 ± 0.18 (7)	0.06 ± 0.05 (7)
John Day	2.39 ± 0.53 (6)	1.40 ± 0.21 (6)	0.25 ± 0.10 (6)
Males			
Estuary	18.93 ± 8.52 (8)	13.90 ± 7.38 (8)	0.10 ± 0.03 (8)
Bonneville	1.14 ± 0.35 (9)	0.92 ± 0.21 (9)	0.05 ± 0.02 (9)
The Dalles	2.09 ± 0.50 (5)	0.72 ± 0.25 (5)	0.01 ± 0.01 (5)
John Day	4.94 ± 2.35 (7)	2.38 ± 0.82 (7)	0.08 ± 0.04 (7)

Table 3. Age, condition factor (CF), relative weight (W_r), and gonadosomatic index (GSI) of legal-size white sturgeon captured in commercial fisheries in the lower Columbia River estuary and reservoirs. Data are means ± sem (n). Different letters denote statistically significant differences between locations.

Location	Age	CF	W_r	GSI
Estuary	14 ± 4 (4) [a]	0.078 ± 0.002 (16) [a]	95.30 ± 1.97 (16) [a]	0.89 ± 0.34 (16)
Bonneville	20 ± 1 (15) [b]	0.065 ± 0.002 (15) [b]	78.39 ± 2.32 (15) [b]	0.45 ± 0.08 (15)
The Dalles	15 ± 1 (13) [a]	0.069 ± 0.001 (13) [bc]	83.15 ± 1.41 (13) [bc]	1.30 ± 0.41 (13)
John Day	17 ± 1 (12) [ab]	0.075 ± 0.002 (13) [ac]	91.01 ± 3.03 (13) [ac]	0.91 ± 0.10 (13)

Figure Captions

Figure 1. Tissue mercury content in legal-size white sturgeon captured in the lower Columbia River commercial fisheries. Data are means ± sem. Different letters denote statistically significant differences between locations. Note the y-axis scale differs.

Figure 2. Reciprocal-Y regression of plasma testosterone and 11-ketotestosterone versus muscle mercury concentration and plasma estradiol versus liver mercury concentration in male and female white sturgeon captured in the lower Columbia River commercial fisheries. Two mature males are denoted by the filled circles in the reciprocal-Y regressions of the androgens and muscle mercury content.

Figure 3. Scatterplot of muscle, liver, or gonad mercury content and plasma testosterone in immature white sturgeon males captured in the lower Columbia River commercial fisheries. The dashed line denotes the possible threshold concentration of mercury affecting steroidogenesis (187 ppb in muscle, 93 ppb in liver, 74 ppb in gonad). Note the x-axis scale differs.

Figure 4. Linear regression of condition factor and relative weight versus mercury concentration in gonad and liver of legal-size male and female white sturgeon captured in the lower Columbia River commercial fisheries.

Figure 5. Linear regression of gonadosomatic index and gonad mercury content in immature male and female legal-size white sturgeon captured in the lower Columbia River commercial fisheries.

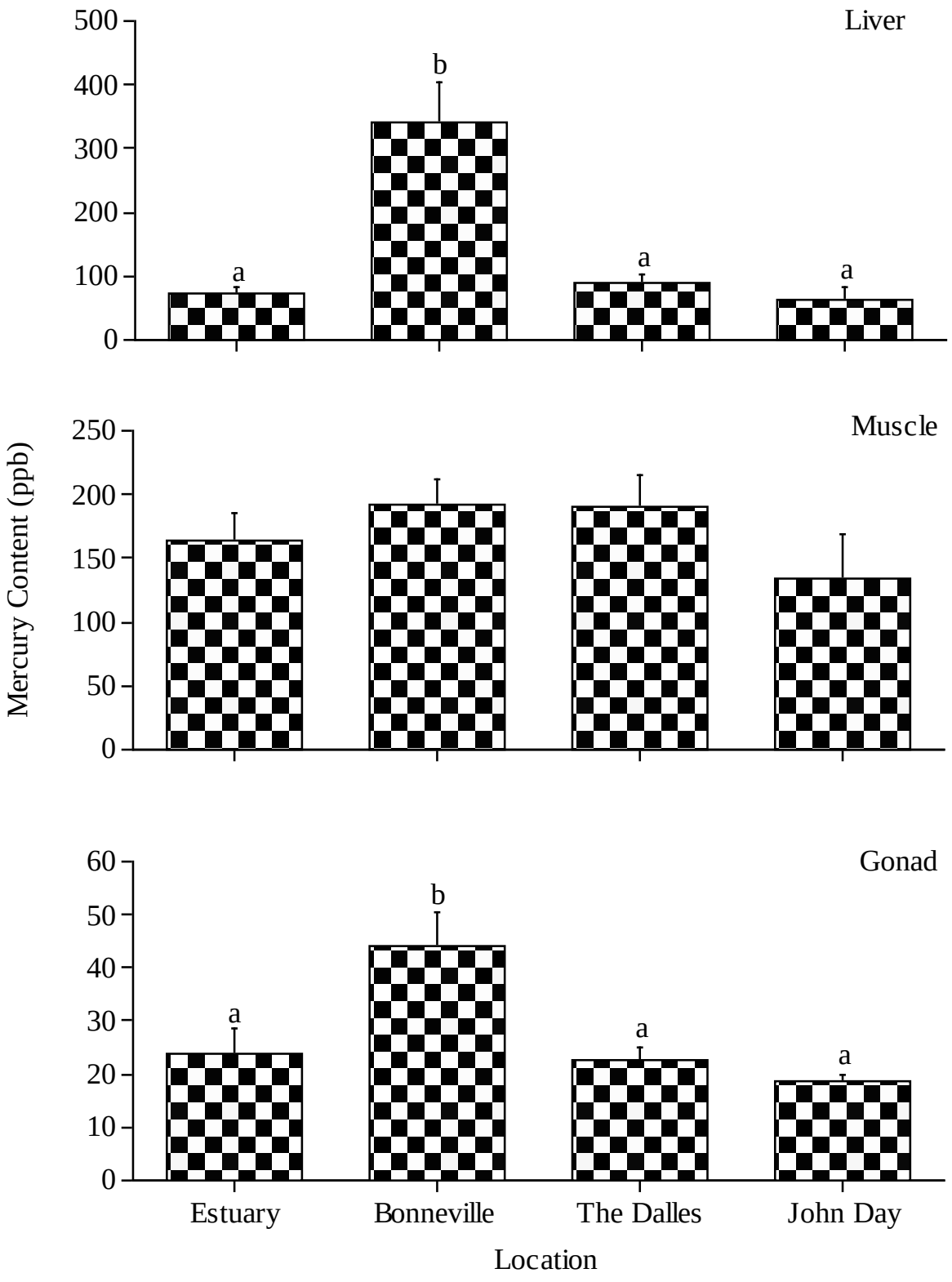
Liver
500
400
300
200
100
0
a
b
a
a
Muscle
250
200
150
100
50
0
Mercury Content (ppb)
Gonad
60
50
40
30
20
10
0
a
b
a
a
Estuary
Bonneville
The Dalles
John Day
Location

Figure 1

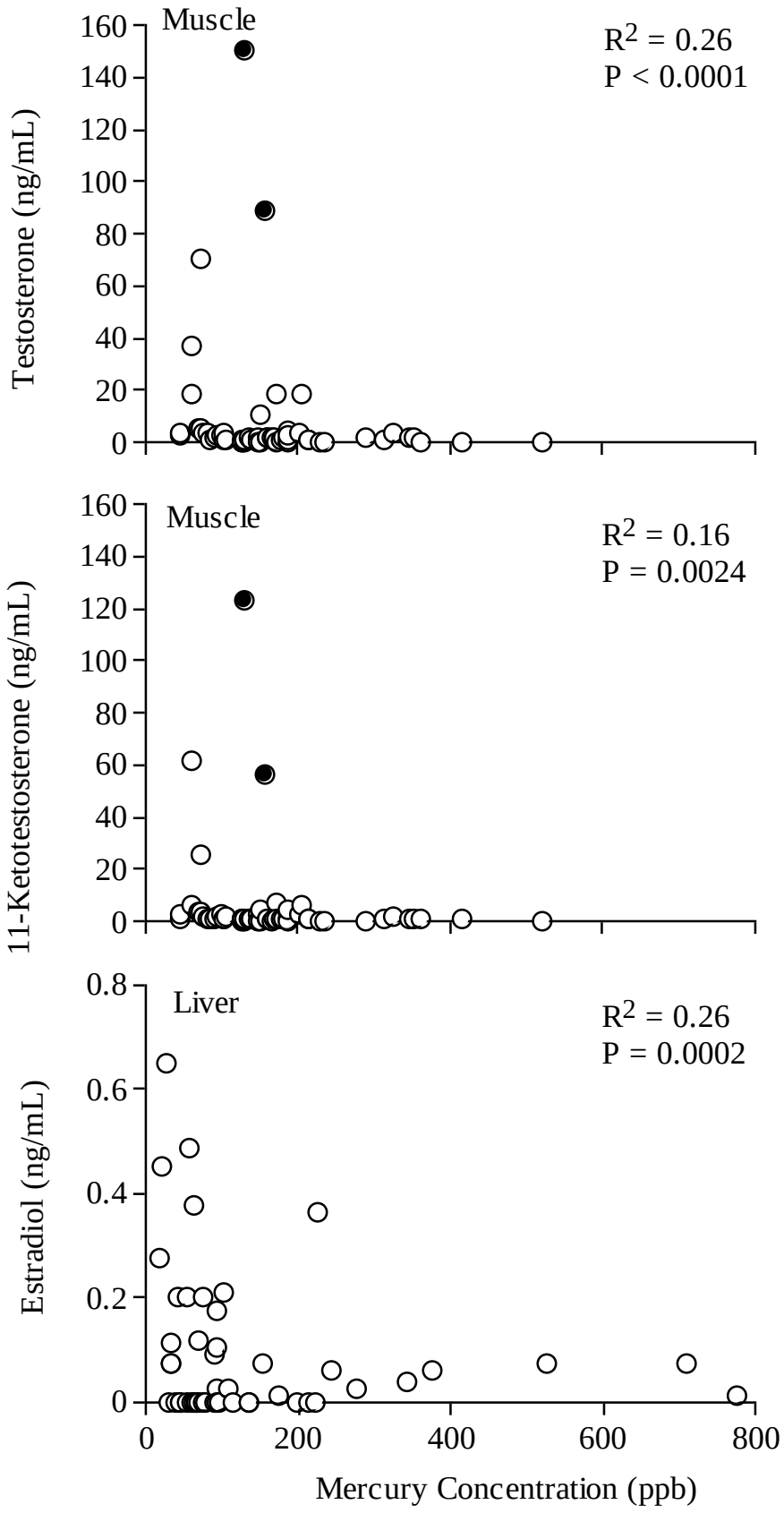
Muscle
R2 = 0.26
P < 0.0001
Testosterone (ng/mL)
160
140
120
100
80
60
40
20
0
Muscle
R2 = 0.16
P = 0.0024
11-Ketotestosterone (ng/mL)
160
140
120
100
80
60
40
20
0
Liver
R2 = 0.26
P = 0.0002
Estradiol (ng/mL)
0.8
0.6
0.4
0.2
0
0
200
400
600
800
Mercury Concentration (ppb)

Figure 2

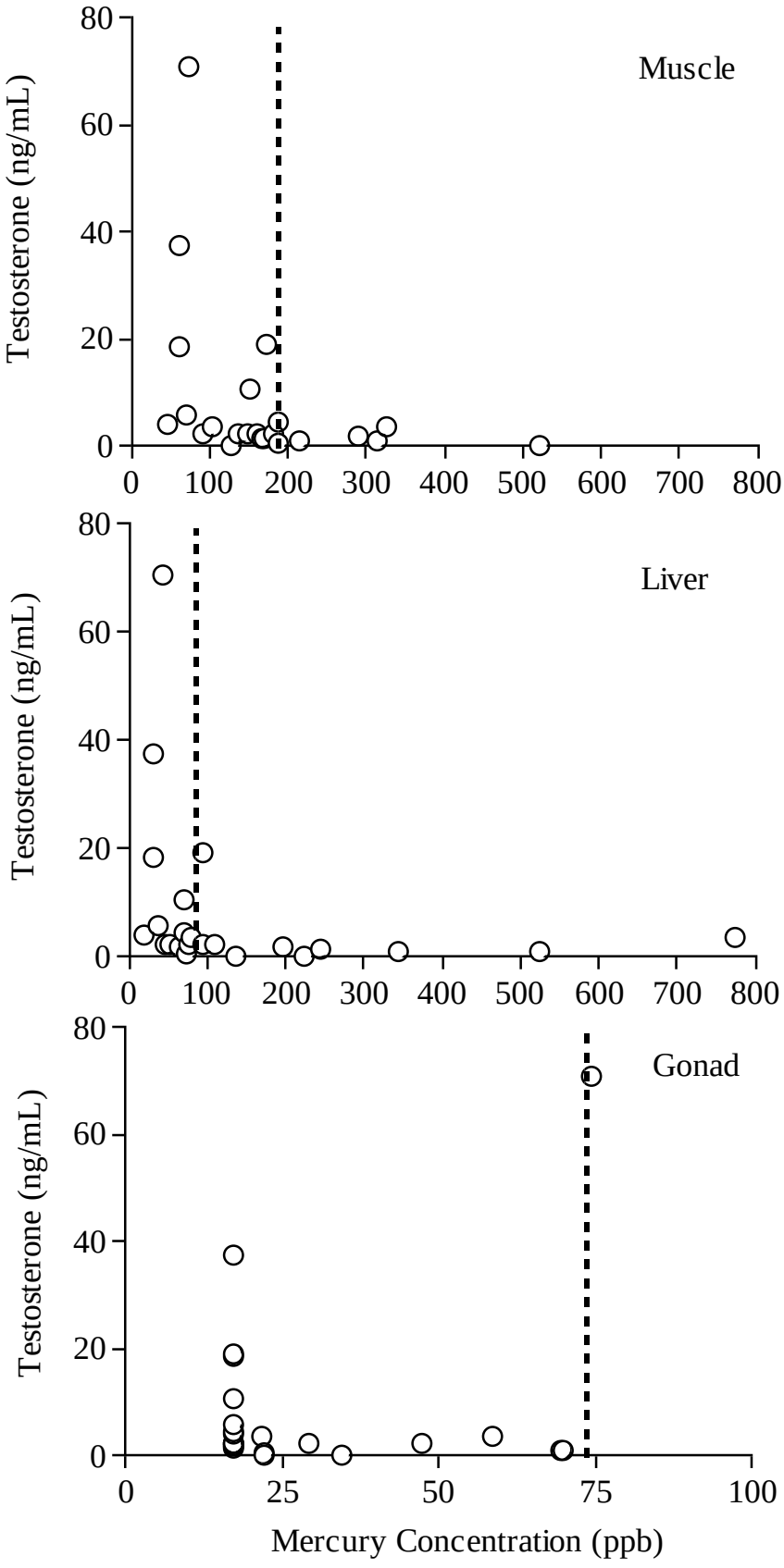
Muscle
Liver
Gonad
Testosterone (ng/mL)
Mercury Concentration (ppb)
0
20
40
60
80
0
100
200
300
400
500
600
700
800
25
50
75

Figure 3

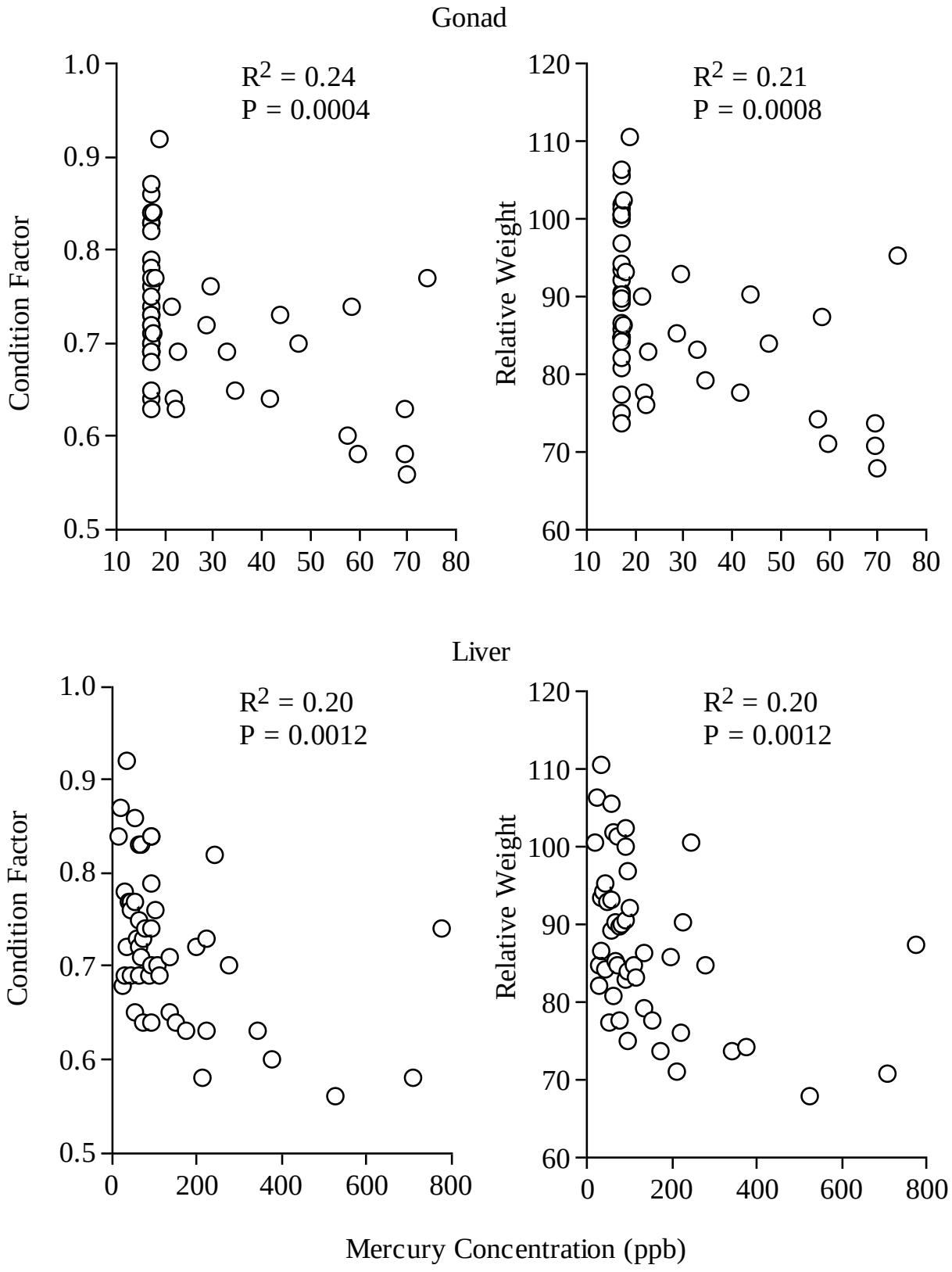
Gonad
R2 = 0.24
P = 0.0004
Condition Factor
1.0
0.9
0.8
0.7
0.6
0.5
10 20 30 40 50 60 70 80
R2 = 0.21
P = 0.0008
Relative Weight
120
110
100
90
80
70
60
10 20 30 40 50 60 70 80
Liver
R2 = 0.20
P = 0.0012
Condition Factor
1.0
0.9
0.8
0.7
0.6
0.5
0 200 400 600 800
R2 = 0.20
P = 0.0012
Relative Weight
120
110
100
90
80
70
60
0 200 400 600 800
Mercury Concentration (ppb)

Figure 4

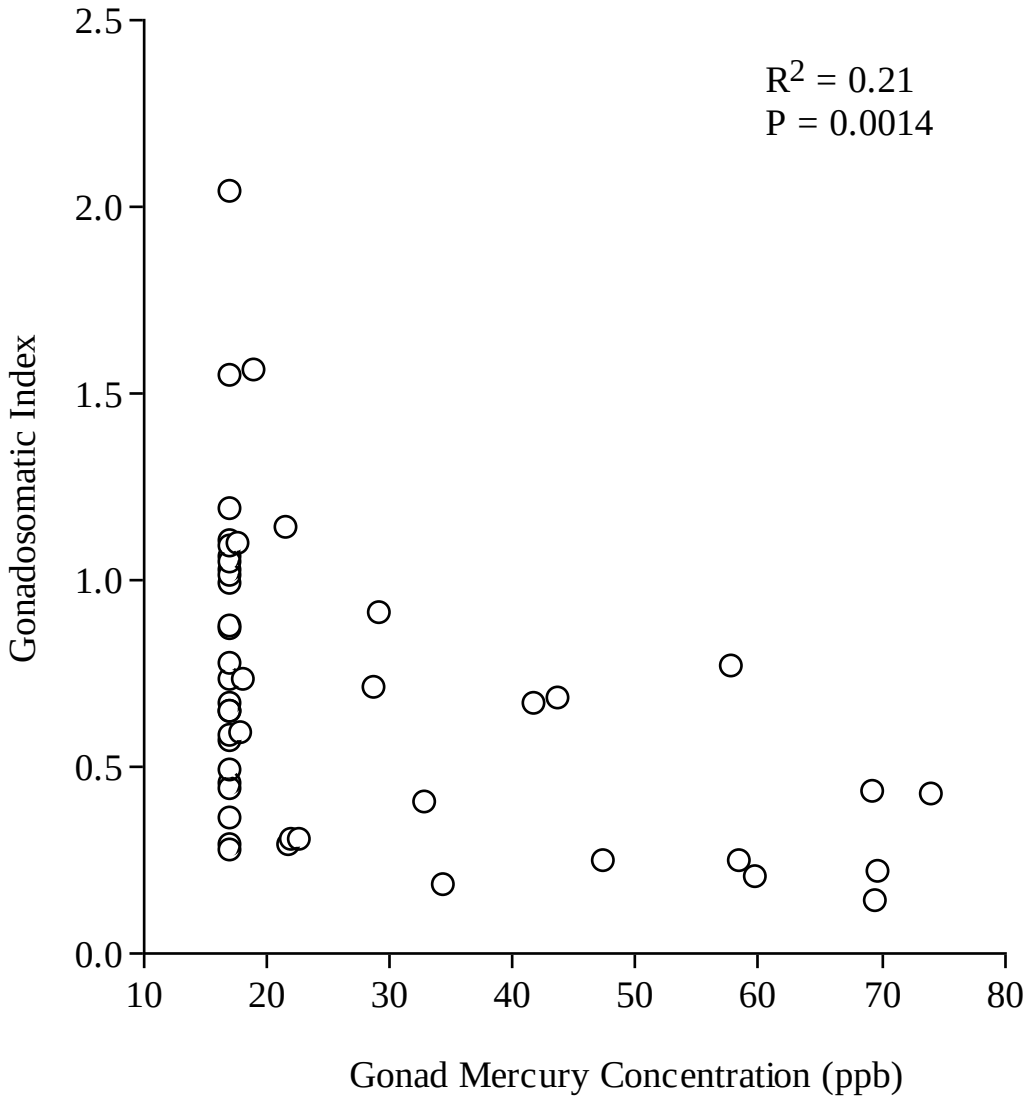
R^2 = 0.21
P = 0.0014
Gonadosomatic Index
2.5
2.0
1.5
1.0
0.5
0.0
10
20
30
40
50
60
70
80
Gonad Mercury Concentration (ppb)

Figure 5